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ADAPTATION OF NEWCASTLE VIRUS TO MAMMALS

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/ Following is the translation of an article by V. N. Syurin, Doctor of Veterinary Science, published in the Russian-language periodical Trudy (Gosud) nauchno-kontrol Inst. vet. Preparatov (Transactions of the Scientific-Control Institute of Veterinary Preparations), No 7, 1957, pages 91--105. Translation performed by Sp/7 Charles T. Ostertag Jr. /

Many investigators have studied the susceptibility of various birds to Newcastle virus and have observed that the age of the birds is one of the main factors influencing susceptibility to experimental infection.

Asplin (1947) established that during experimental infection of ducks and geese with this virus some remained asymptomatic, but were infectious for chicks for a period of 6--8 days.

Haddow and Idnam (1946) passaged the pseudoplague virus through ducks. One to ten day old birds became infected by intramuscular and subcutaneous inoculation and the majority survived. The authors worked with three strains and observed that one of these, following the 5th passage, became attenuated for chickens and multiplied more slowly in chick embryos, causing their death in 3--7 days. Though after 2--3 successive passages in chick embryos, normal multiplication and lethality of the virus for embryos was restored, its virulence for birds remained low. Subsequently this strain was cultivated in chick embryos and maintained its attenuation for chickens. When it was used as a vaccine on a healthy bird, the loss of the latter as a result of vaccination fluctuated within the limits of 0.9 to 3.1%.

Komarov and Goldsmit (1946) passaged the Palestine strain of Newcastle virus in the brain of ducklings and obtained a stable biological attenuation for ducks. The virus was attenuated comparatively rapidly during intracerebral passaging in ducklings. The virus containing brain of the 10--14th passage was already avirulent for young chicks following inoculation in the wattle.

Reagan, Lillie and Brueckner (1947, 1948), beginning in 1944, conducted investigations on the experimental attenuation of Newcastle virus by serial passaging through Syrian hamster brains.

California strain No. 11,914, isolated in 1944 during a natural outbreak of Newcastle disease, was passaged 22 times in chick embryos. The titer for embryos during passage rose to 0.1 ml x 10^{-8}. Virus of the last embryo passage was transferred to 4-week old hamsters. Virulence for hamsters increased, which is apparent from the following: In the first 50 passages of the Dlm following cerebral inoculation, the lethal dose was 0.08 ml of a 10% brain suspension. From the 50th through the 100th passage it dropped to 0.03 ml

(with the same concentration of brain), and from the 150th up to the 330th passage the virus caused 100% death of animals with a dose of 0.03 ml (1% concentration). The titer of the brain suspension of the 330th passage reached $10^{-4.5}$ (Reagan and Brueckner, 1954). The authors studied the clinical picture of the disease in hamsters.

The adaptation of California strain (11,914) to hamsters considerably increased its infectivity for other animals.

In 1950 Reagan, Smith and Bruecker passaged this virus 6 times by intracerebral inoculation of bats (Myotis licifugus). In further adaptation the virus caused fatal illness in bats even by intranasal inoculation. Reagan and others (1951) conducted 6 passages with strain 11,914, but during the simultaneous passaging of 6 other strains, not adapted to the hamster, negative results were obtained.

Foreign literature contains many reports of experimental infection of rhesus monkeys (Macaca mulatta) with the adapted California strain of the virus. Thus Reagan, Werner and Bruecker (1950), using virus of the 310th hamster passage, successfully infected the rhesus monkey by the intracerebral route and in this manner passaged the virus 10 times.

Experiments in passaging the adapted California strain of the virus in weasels, rabbits and guinea pigs were unsuccessful (Reagan, Lillie, 1947; Reagan, Werner, Schenk, 1950). Weasels, rabbits, guinea pigs and hamsters, inoculated intracerebrally with the 300th passage of the hamster adapted California strain, displayed similar symptoms of the disease, however, during successive passages the virus disappeared from all animals (except the hamsters). Subsequently Reagan and others (1954) made simultaneous tests on rabbits with 6 strains of Newcastle virus which were adapted to chick embryo. They observed that only young rabbits were susceptible to this virus. In comparison with the other five strains, California strain 11,914 after 300 passages in Syrian hamsters and 33 passages in embryos was more virulent for rabbits following intracerebral inoculation.

With the virus from the 16th hamster passage it was possible to cause illness in 30% of the infected guinea pigs, however subsequent passage of the virus in this species was unsuccessful.

It was possible to transfer the hamster adapted California strain to white mice. With virus from the 8th hamster passage, Reagan infected young mice and passed it four times. Further adaptation of the virus to hamsters was accompanied by an increased capacity to develop in the brain of white mice (Reagan et al. 1949; Reagan, Bruecker, 1951).

Our investigations on the alteration of the biological properties of the avian pseudoplague virus were begun in 1947. They were an attempt to attenuate a virulent epizootic strain of the Newcastle virus by adaptation.

Initially no data were available on the immunological and antigen differences of the epizootic strains isolated in the USSR. On the other hand, the

investigations by M. D. Polykovskiy, I. N. Doroshko, P. M. Svintsov, A. Ya. Fomina and others suggested identity of properties of pseudoplague strains isolated in various geographic zones of the USSR. We arbitrarily selected the epizootic strain "T" (Tomilinskiy), isolated in 1946 during an outbreak of pseudoplague at the Tomilinskiy Poultry Plant.

In the period from 28 January 1947 through 15 May 1947, this strain was passaged 22 times in chick embryos. It not only increased in virulence for embryos (logLD_{50} comprised 11.7), but also for birds. The "embryonic" virus of 2, 4, 10, 13, 17 and 22 passages in doses from 1:500x0.5 ml to 10^{-5}x0.5 ml, as a rule, caused the death of all the infected birds with the typical lesions of avian pseudoplague.

Having increased the virulence of this strain for embryos, we shifted to adapting it to ducks. Since preliminary tests showed that mature ducks did not become sick following intracerebral inoculation with strain "T", we began the experiments with ducklings and the last passages were made with mature ducks.

Infected ducklings and ducks, as a rule, became ill in 48--72--120 hours with the same clinical picture of central nervous system disease. Thirty intracerebral passages were carried out. Testing the brain of dead ducklings in poultry (21 tests) showed that the virus, as a result of such cultivation, hardly lowered its virulence for poultry, causing death in 91 out of a 100 cases. The inoculated poultry, as a rule, died in periods which were usual for the natural virus of avian pseudoplague, however, the virus caused a disease in the infected poultry which was clinically different from the disease during infection with the epizootic strains "T", "U" and "K"; signs of central nervous system disease prevailed in the syndrome of the sick poultry.

The second phase of our investigations consisted of the transfer of the duckling adapted virus to guinea pigs. From September 1948 to December 1956 we passaged the virus in guinea pigs and other mammals. The first 23 passages of the virus through the brain of guinea pigs showed that the virus from dead guinea pigs caused the illness not only of chickens, but also of rabbits, guinea pigs and sheep.

Subsequently, beginning with the 23rd guinea pig passage, the cultivation of the virus continued along two parallel lines. The first line of direct successive passages was conducted in guinea pigs. For 8 years the virus of this line passed through the brain of guinea pigs 214 times. The presence of virus in the brain of sick guinea pigs was supported not only by infection of embryos, but also of other animals (rabbits, kittens). Beginning with the 72nd and 159th direct passage, the virus from the guinea pigs was passaged in parallel in chick embryos, and in this way the first and second "embryonic" passage lines emerged.

The second line of tests (beginning with the 23rd generation) consisted of passing the virus through guinea pigs, sheep, rabbits and mice by means of alternate passaging. It may be observed that the virus of the line of alternate passages was passed three successive times in sheep (24--26th passages). After 21 passages through guinea pigs it was again passed through sheep (28 passages);

51 passages of this virus were also conducted in sheep, and the 96th and 99th passage -- in rabbits. Beginning with the 103rd through the 124th passages, the virus passed a number of alternate generations between guinea pigs and white mice.

Subsequent passaging of this virus line was conducted in guinea pigs, with the exception of alternate passages (110--156) in guinea pigs - embryos. We tested the virus of the line of alternate passages in cats, sheep and rabbits. By the beginning of March 1957 this virus line had gone through 240 alternate passages.

On 1 December 1956 in tests of the "direct" line of passages, 920 guinea pigs were infected and of these 585 died (63.4%). In the parallelly conducted alternate 220 passages, 732 guinea pigs were used. Of these, 465 animals (63.5%) died. We infected 148 white mice and .3 (36%) died. The virus was tested in 18 rabbits and 17 kittens. In the tests of the alternate passages, 10 sheep were used. Of these, 8 animals died.

In contrast to the data of Reagan and Lillie (1947), in whose tests, beginning with the 8th passage, the death of all infected hamsters occurred, in our tests the lethality of infected guinea pigs varied in rather wide limits; in the "direct" line of passages 45%--92%, and in the line of "alternate" passages 36.6%--100%.

The number of tests on infecting animals in each passage was also different. In a number of cases, particularly in the tests of 1950, an average of 1.1 and 1.3 tests of infection were used for each passage, while in 1954 for carrying out one passage of the virus 2.4 and 3.4 tests of infection were required.

Table 1 shows the variation in the ratio of the number of tests of guinea pig infection conducted to the number of passages made.

We cannot adequately explain the reason for such a wide variation in the percentage of lethality of infected animals, nor the difference in the relationship of the number of tests of animal infection to the number of passages performed.

The clinical picture of the disease in the infected guinea pigs was characterized by a diversity of signs. The incubation period varied from 3 to 7 days and rarely to 9 days. In the first 2--3 days following the injection of the virus the pigs seemed completely healthy. Then the signs of central nervous system disease appeared. The sick pigs became sluggish and sat with ruffled fur. After 10--20 hours this condition changed to irritability; the animal stood up on its hind legs and scratched its nose and sides. It was noted that every 15--20 minutes the animal jerked its head to one side. Sometimes this was accompanied by a slow raising of the head while the animal remained sitting for 10--15 seconds gritting its teeth or slowly retracting its head with spasmodic back bending. This was usually accompanied by a slight muscle tremor and gritting of teeth. Accumulation of saliva was noted in the corners of the mouth during these attacks. The amount increased with time.

During the first attack of the disease, along with the above described signs, guinea pigs displayed severe muscle spasms (rigidity) of the front and rear limbs, neck and back (figure 2). In this condition the pig often turned to one side or fell on its back (figure 3).

These signs of the disease were followed by weakness, depression and complete prostration. The pig became indifferent to the surroundings, did not react to pricking and jerking, and sat sullenly with half colsed eyes (figure 4).

Sometimes the disease began without visible indications of involuntary movement of the jaws or spasmodic jerking of the head. The pig, which appeared normal, unexpectedly began to walk in circles, striking obstacles and animals. Such walking in any direction was accompanied by a spasmodic bending and simultaneous sudden twitching of the head. In some animals these signs were replaced by extreme irritability and convulsions. The fits of irritability were replaced by depression: The animal became sluggish and apathetic, and tried to force itself into a corner of the cage or crawl under healthy animals. Then locomotor disorders and paralysis of front and hind limbs developed. Death occurred 8--18 hours after the onset of signs.

The reaction of the sick guinea pigs was manifested not only by clinically apparent signs of disease of the central nervous system, but as a rule was also accompanied by changes in weight and body temperature.

The incubation period of the disease for affected mice varied from 3--11 days. Approximately 84% of the infected mice became sick from the 4th through 7th day.

The clinical picture of experimental infection in mice was characterized by a diversity of signs. From time to time there was head jerking, sometimes the animal stood on its hind legs swaying from side to side, or leaned its head backwards until it fell on its back. Sometimes a mouse, standing on its hind legs, made involuntary rhythmic jerks with its head to one side. Often this was replaced by severe irritability, leading to a convulsion. Right after this depression usually set in, along with a loss of reactivity to external stimulation, paralysis and death.

As a rule, infected kittens became ill in 5--7 days. Typical signs of the disease in kittens: Jerking of the head, circus movements, salivation, mewing sounds and profuse salivation.

The adapted virus caused disease in other rodents also (rabbits, susliks), and produced an unique clinical picture, similar to the one described above, of a functional disorder of the central nervous system (figures 5, 6).

From the description of the clinical illness in experimentally infected animals, it is seen that Newcastle virus, as a measure of adaptation to the central nervous system of rodents, caused a disease with an abbreviated incubation period and severe clinical picture of a central nervous system disease.

Clinically, animals inoculated intracerebrally with the original virulent strain "T", exhibited more severe central nervous system depression than from the adapted virus. In pigs which became ill from the unadapted strain "T" symptoms of severe depression were noted: The pigs sat sullenly, their fur lost its luster and became ruffled, anorexia developed and usually death followed prostration without the periodic attacks of stimulation of the central nervous system as observed with the adapted virus.

The presence of the virus in the organs and tissues of sick and dead guinea pigs was determined by inoculating developing chick embryos. Brains of animals dying from direct line of passage virus were tested 36 times, and the alternate line -- 28 times. In all cases virus containing brains of dead pigs killed embryos at a dose of 1:20x0.15 ml in 48--70 hours. With three successive tests it was proven that the brain of infected guinea pigs, not displaying signs of illness, also contained the active virus.

The titer of "brain" virus for the embryos varied, and apparently depended on the number of guinea pig brain passages, which is apparent from table 2.

Inoculation of guinea pigs subcutaneously, intranasally and intramuscularly did not produce disease. Following the intracerebral inoculation of pigs the virus in dead animals was detected only in the hemispheres of the brain and the cerebellum. Virus was not detected in the medulla oblongata, medulla ossium, blood, lungs and urine. However, on the basis of these tests it was not possible to conclude viremia was absent during the course of experimental infection, since during other viral infection viremia of short duration was observed which was absent at time of death.

Considering this fact, we conducted a series of tests for establishing the time of the appearance and disappearance of the adapted Newcastle virus in the blood of infected guinea pigs. In the tests we used virus 72/78 and 72/81, that is, viruses which had undergone 72 passages in guinea pigs and 78 and 81 passages in embryos. In respect to passaging in embryos, this virus possessed encephalogenic properties following intracerebral inoculation, but was avirulent following intranasal, intramuscular and subcutaneous administration to guinea pigs.

The presence of viremia was established in guinea pigs, infected with the 72/78 virus by the intramuscular route. With this aim we conducted two orientation and three repetitive tests with titration of the virus in embryos.

On 19 May 1954, two guinea pigs (NoNo 368 and 513) were inoculated intramuscularly with the 72/78 virus. Virus containing allanto-amniotic fluid was inoculated in the muscles of the femur in a dose of 10^{-1} x 1.0 ml. Simultaneously as a control, two guinea pigs (NoNo 388 and 349) were infected intracerebrally with the same virus. On 24 May 1954 the control animals became ill with the typical clinical picture of encephalitis and died on the following day; cultures from the organs and brain did not contain bacteria.

The test pigs remained healthy for a period of 10 days. In 24, 48 and 72 hours following infection, blood was taken from their hearts. It was

hemolyzed with sterile distilled water (1:1) and immediately inoculated intracerebrally to guinea pigs in doses of 0.2 ml. Part of the animals which had become ill and died from the hemolyzed blood were subjected to bacteriological investigation, and their brains were tested for the presence of the virus by inoculating developing chick embryos. The brain suspension from the dead guinea pigs was inoculated onto the chorioallantoic membrane. The results of this test are presented in a summarized form in table 3.

In order to establish that the embryonic material (allanto-amniotic fluid) contained a virus, encephalogenic for guinea pigs, on 8 July 1954 six guinea pigs were inoculated. With a mixture of sterile samples of embryonic fluids, corresponding to pigs No 358 and 336, animals were inoculated by the intracerebral route with doses of 10^{-2} x 0.2 ml each. The results are shown in table 4.

Subsequently two more tests were conducted (20 and 28 July 1954) to detect virus in the blood of five guinea pigs infected intramuscularly with the 72/81 virus.

In the first test the blood was taken from the heart of three infected guinea pigs (NoNo 2764, 3982 and 1710) after 24, 48, 72, 96 and 120 hours, mixed, hemolyzed by the addition of sterile distilled water in the ratios of 1:1, 1:5, 1:10, 1:50, 1:100, 1:1000 and 1:10000, and tested in 10-day old embryos. The embryos were inoculated by the chorio-allantoic route. Six embryos were infected with each dilution of the material.

In the second test the same method was used for testing the blood of two guinea pigs infected intramuscularly with the same virus in a dose of 1:10 x 1.0 ml.

Summarized results of the titration in embryos are presented in table 5.

Thus, following the intramuscular inoculation of guinea pigs it is possible to isolate embryo adapted virus 72/78 and 72/81 from the blood between 24 and 72 hours. After 48 hours its content in the blood fluctuates within the limits of 1.69 and 2.5 $\log LD_{50}$. After three days the concentration of virus in the blood was insignificant (0.40 $\log LD_{50}$), and after four days it was not possible to detect it in the blood by biological methods.

The tests showed that following the administration of adapted Newcastle virus to guinea pigs by the intramuscular route it was not possible to cause illness. Following such an administration the guinea pig remained naturally non-susceptible, which is apparently explained by the disparity of exchange of cellular substances of the various tissues of the organism-host to the needs of the virus. In subsequent tests this natural antiviral resistance following intramuscular, intranasal, and subcutaneous inoculation could not be overcome either by increasing the dose or by weakening the general resistance of the guinea pig. Following intracerebral inoculation of the virus to the same pigs, the virus not only was fixed but also reproduced in the cells of the brain, causing a clinically expressed disease.

However, if during the period of viremia (following the intramuscular

administration of the virus) the brain is traumatized by the administration of an 0.2 ml physiological solution, then the disease develops. This was proven by direct tests on five guinea pigs.

Conclusions

1. Strain "T" of the Newcastle virus was adapted to guinea pigs and other mammals. Following the intracerebral inoculation, the adapted virus caused an infection, clinically expressed by signs of central nervous system disease (irritability, anorexia, locomotor disruption, paralysis).

2. In dead guinea pigs the virus was detected in the cerebrum and cerebellum. In guinea pigs which were infected intramuscularly the presence of the virus in the blood (viremia) was proven by the biological method between 24--72 hours after inoculation.

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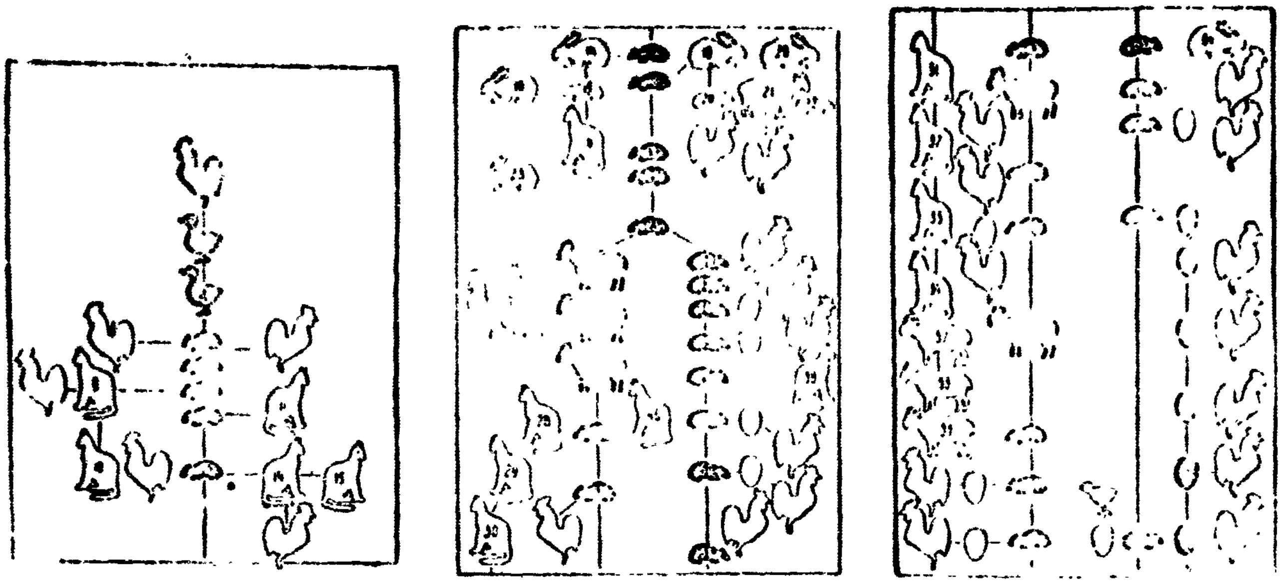
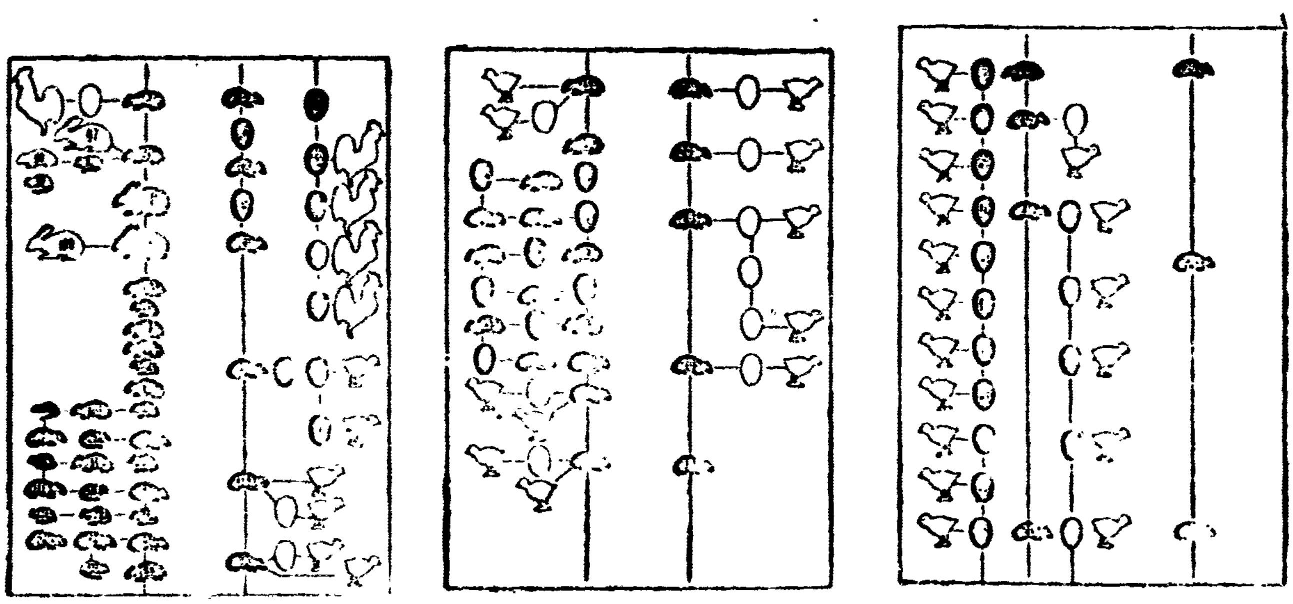


Figure 1. Passages of Newcastle virus in animals



Passages of Newcastle virus in animals.

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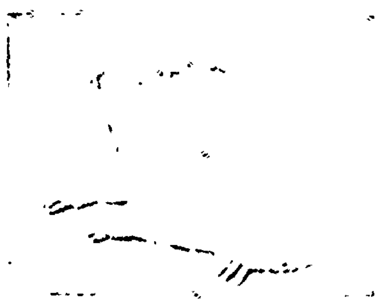

Figure 2. Guinea pig No 395, which received intracerebrally 0.2 ml (1:25) of adapted pseudoplague virus. Onset of nervous fit. Front extremities stretched out forward, head thrown back and turned somewhat to the right. Eyes opened widely.

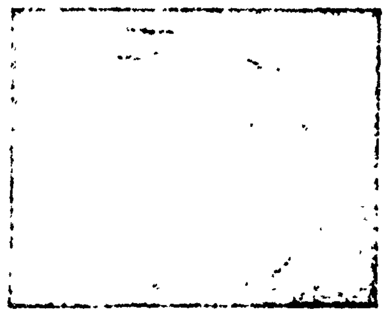

Figure 3. Guinea pig No 395. Signs of irritation of the central nervous system. Pig is twisted through the back.

Figure 4. Guinea pig No 395. End of convulsion. Manifestation of strong depression, eyes half closed.

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GRAPHIC NOT REPRODUCIBLE

Figure 5. Rabbit No 312. End of convulsion. Symptoms of depression. Weakening of the muscles of the pelvis, sacrum and neck.

GRAPHIC NOT REPRODUCIBLE

Figure 6. Rabbit No 312. Strong depression. Complete weakness of the muscles of the entire body of the animal. Position of "cross."

Table 1

Relationship of the number of tests on the infection of animals to the number of passages

Year	Line of direct passages			Line of alternate passages		
	Number of passages	Tests of infection performed	Ratio of number of tests of infection to number of passages	Number of passages	Tests performed	Ratio of number of tests of infection to number of passages
1948	9 (1--9)	15	1.7	--	--	--
1949	11 (10--20)	16	1.45	--	--	--
1950	18 (21--38)	23	1.3	24 (23--46)	27	1.1
1951	27 (39--65)	36	1.3	28 (47--74)	36	1.3
1952	20 (66--85)	40	2.0	32 (75--106)	42	1.3
1953	32 (86--117)	45	1.4	35 (107--141)	51	1.4
1954	23 (118--140)	55	2.4	21 (142--162)	72	3.4
1955	48 (141--189)	51	1.0	34 (163--197)	42	1.2
1956	24 (190--214)	25	1.0	22 (198--220)	24	1.2

Table 2

Titer of "brain" virus for embryos

Virus (passage)	No. of guinea pig	Titer
120th alternate	577	5.15
120th alternate	549	5.16
159th direct	366	4.58
167th alternate	408	3.63

Table 5

Presence of virus in the blood of guinea pigs, infected intramuscularly

Testing of blood, obtained after	Log LD_{50} in the tests	
	First	Second
24 hours	0	0
48 hours	1.69	2.50
72 hours	0.40	0.40
96 hours	0	0
120 hours	0	0

Table 3

Presence of virus in the blood of guinea pigs following intramuscular inoculation

Blood taken from guinea pigs	Blood taken after inoculation (hrs)	Number of inoculated guinea pigs	Onset of illness	Clinical picture of disease, date of death or slaughter of animal, sterility of cultures from organs of the animal	Testing of material on embryos				
					Date of infection	No of embryo	Term of death (in hours)	Pathological changes	Sterility of cultures
368 and 513	24 h (20/V 1954)	701 361	— —	Did not become sick during the course of 20 days of observation	— —	- -	— —	— —	— —
368 and 513	48 h (21/V 1954)	358	26/V 1954	Clinical signs of encephalitis noted. Slaughtered in state of agony 27/V 1954. Cultures sterile	3/VI 1954	1 2 3 4 5	48 60 72 72 72	++ + + ++ ++	Ster. " " " "
368 and 513	48 h (21/V 1954)	336	26/V 1954	Clinical signs of encephalitis noted. Died 26/V 1954. Cultures sterile	3/VI 1954	1 2 3 4 5	60 60 40 72 72	++ ++++ ++++ + ++	Growth Ster. " " "
368 and 513	72 h (22/V 1954)	503	26/V 1954	Nervous symptoms noted, death at night 27/V 1954. Brain flaccid. Cultures yielded growth	—	-	—	—	—
		325	—	Did not become sick during the course of 10 days of observation	—	-	—	—	—

Table 4

Presence of virus in the brain of guinea pigs which had died from virus containing blood obtained after 48 hours

Embryonic virus being tested, obtained from multiplication of guinea pig viruses	NoNo of inoculated pigs	Onset of illness	Clinical picture, data of death of the animal	Sterility of cultures
No 358	7264	14/VI 1954	Typical clinical picture of encephalitis. Died 14/VI 1954	Brain--sterile Heart--sterile
	3427	12/VI 1954	Depression, paresis of forward extremities. Died 3/VI 1954	Brain--sterile Heart--sterile
	316	12/VI 1954	Depression, locomotor disorders. Died 14/VI 1954	Brain--sterile Heart--growth
No 336	824	12/VI 1954	Same. Died 12/VI 1954	Brain--sterile Heart--sterile
	3729	13/VI 1954	Agitation, clinical picture of illness with encephalitis. Died 13/VI 1954	Brain--sterile Heart--sterile
	8	12/VI 1954	Same. Died 14/VI 1954	Brain--growth Heart--sterile